

AMENDMENTS TO THE CLAIMS

The following listing of claims, in which Applicants amend claim 4, will replace all prior versions and listings of claims in the application.

Listing of Claims:

1-3. (Canceled)

4. (Currently amended) A compound having a formula selected from

V-(W-)_w(X-)C((A-)_aZ)_c,

V-(W-)_w(X-)C(D((A-)_aZ)_d)_c,

V-(W-)_w(X-)C(D(E((A-)_aZ)_e)_d)_c, and

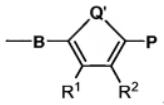
V-(W-)_w(X-)C(D(E(F((A-)_aZ)_f)_e)_d)_c,

wherein:

V is an enzymatically removable specifier comprising an optionally protected peptide, which is optionally removable after binding to a receptor, or

taken together, **V-B** is an oxidized form of **B**, wherein **B** is part of **C-W** or **X**;

each of **W** and **X** independently is a single release 1,(4+2n) electronic cascade spacer and has the formula:



wherein

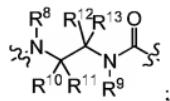
Q' is—R⁵C=CR⁶—;

B is selected from NR⁷, O, and S;

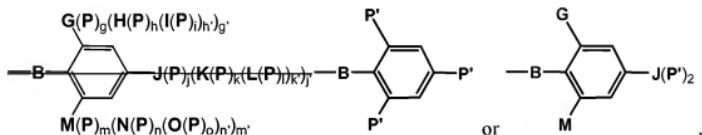
P is C(R³)(R⁴)Q;

Q is $-O-CO-$;

A is an ω -amino aminocarbonyl cyclization elimination spacer having the formula:



each of **C**, **D**, **E**, and **F** independently is a self-eliminating multiple release spacer or spacer system and has the formula:



wherein

B is selected from NR¹, O, and S;

$[[P]]P'$ is $C(R^2)(R^3)Q-(W-X)_n$; wherein

Q is $-O-CO-$; and

W and **X** are as defined above;

G, **J**, and **M** independently are **P**, and g , h , i , h' , g' , j , k , l , k' , j' , m , n , o , m' , and n' all equal 0; or

G and **M** are hydrogen $[[J]]$; and g , h , i , h' , g' , m , n , o , n' , and m' all equal 0,

J is



and $j=2$ and $j'=0$;

wherein R¹, R², R³, R⁴, R⁵, R⁶, and R⁷ independently are selected from H, a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group, a C₅₋₂₀ aryl group, a C₁₋₆ alkoxy group, hydroxy (OH), amino (NH₂), mono-substituted amino (NR_xH), di-substituted amino (NR_x¹R_x²), nitro (NO₂), halogen, CF₃, CN, CONH₂, SO₂Me, CONHMe, a cyclic C₁₋₅ alkylamino group, imidazolyl, a C₁₋₆ alkylpiperazinyl group, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate (COOR_x), sulphonyl (S(=O)₂OH), sulphonate (S(=O)₂OR_x), sulphonyl (S(=O)₂R_x), sulphoxy (S(=O)OH), sulphinate (S(=O)OR_x), sulphinyl (S(=O)R_x), phosphonoxy (OP(=O)(OH)₂), and phosphate (OP(=O)(OR_x)₂), wherein R_x, R_x¹ and R_x² are independently selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group and a C₅₋₂₀ aryl group;

R⁸ and R⁹ independently are selected from H and C₁₋₆ alkyl, said alkyl being optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_x), amino (NH₂), mono-substituted amino (NR_xH), di-substituted amino (NR_x¹R_x²), nitro (NO₂), halogen, CF₃, CN, CONH₂, SO₂Me, CONHMe, cyclic C₁₋₅ alkylamino, imidazolyl, C₁₋₆ alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate (COOR_x), sulphonyl (S(=O)₂OH), sulphonate (S(=O)₂OR_x), sulphonyl (S(=O)₂R_x), sulphoxy (S(=O)OH), sulphinate (S(=O)OR_x), sulphinyl (S(=O)R_x), phosphonoxy (OP(=O)(OH)₂), and phosphate (OP(=O)(OR_x)₂), where R_x, R_x¹ and R_x² independently are selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group and a C₅₋₂₀ aryl group; and

R¹⁰, R¹¹, R¹², and R¹³ independently are selected from, C₁₋₆ alkyl, C₃₋₂₀ heterocyclyl, C₅₋₂₀ aryl, C₁₋₆ alkoxy, hydroxy (OH), amino (NH₂), mono-substituted amino (NR_xH), di-substituted amino (NR_x¹R_x²), nitro (NO₂), halogen, CF₃, CN, CONH₂, SO₂Me, CONHMe, cyclic C₁₋₅ alkylamino, imidazolyl, C₁₋₆ alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate (COOR_x), sulphonyl (S(=O)₂OH), sulphonate (S(=O)₂OR_x), sulphonyl (S(=O)₂R_x), sulphoxy (S(=O)OH), sulphinate (S(=O)OR_x), sulphinyl (S(=O)R_x)

(S(=O)R_x), phosphonoxy (OP(=O)(OH)₂), and phosphate (OP(=O)(OR_x)₂), wherein R_x, R_x¹ and R_x² independently are selected from a C₁₋₆ alkyl group, a C₃₋₂₀ heterocyclyl group and a C₅₋₂₀ aryl group; or

alternatively, two or more of R⁸, R⁹, R¹⁰, R¹¹, R¹², and R¹³ are connected to one another to form one or more aliphatic or aromatic cyclic structures;

each Z is independently a therapeutic or diagnostic moiety;

a is 0 or 1;

c, d, e, and f are independently an integer from 2 (included) to 24 (included);

w and x are independently an integer from 0 (included) to 5 (included); and

n is an integer of 0 (included) to 10 (included).

5-6. (Canceled)

7. (Previously presented) The compound according to claim 4, wherein the Z groups are linked to the self-eliminating multiple release spacer or spacer system via an O, S, or aromatic N of the Z group.

8. (Previously presented) The compound according to claim 4, wherein the Z groups are linked to the self-eliminating multiple release spacer or spacer system via an aliphatic N and wherein at least one multiple release spacer or spacer system of either generation C, D (if present), E (if present), or F (if present) is a phenol- or thiophenol-based multiple release spacer or spacer system, meaning that

i) B = O or S for at least one multiple release spacer in said generation, or

ii) when B = N for all multiple release spacers in said generation, at least one single release spacer is connected to at least two branches of at least one multiple release spacer in said generation, and B = O or S for at least two of said single release spacers.

9. (Previously presented) The compound according to claim 8, wherein B = O or S for all multiple release spacers or spacer systems in said generation.

10. (Previously presented) The compound according to claim 8, wherein the phenol- or thiophenol-based multiple release spacers are connected to either A or Z.

11. (Cancelled)

12. (Previously presented) The compound according to claim 4, wherein group A is present, and Z is coupled via its hydroxyl group to A.

13. (Cancelled)

14. (Previously presented) The compound of claim 4 wherein

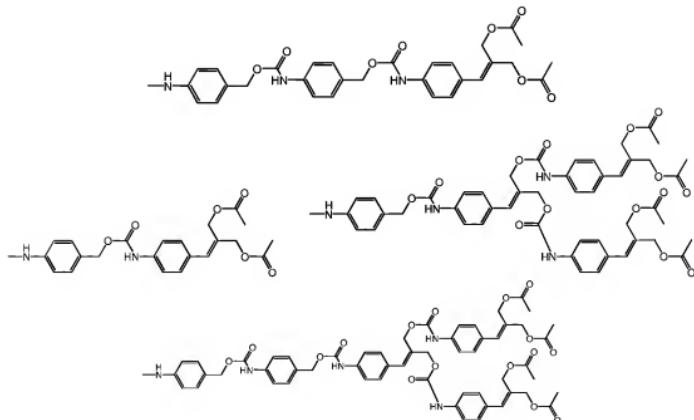
$(W)_w(X-C_c,$

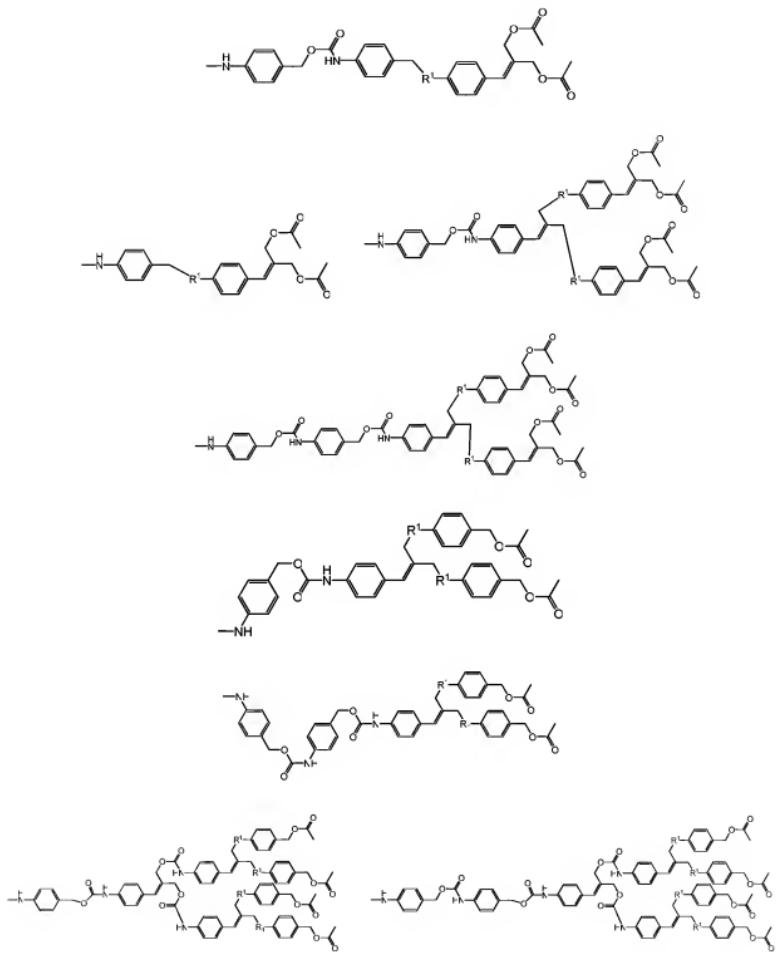
$(W)_w(X-C(D_d)_c,$

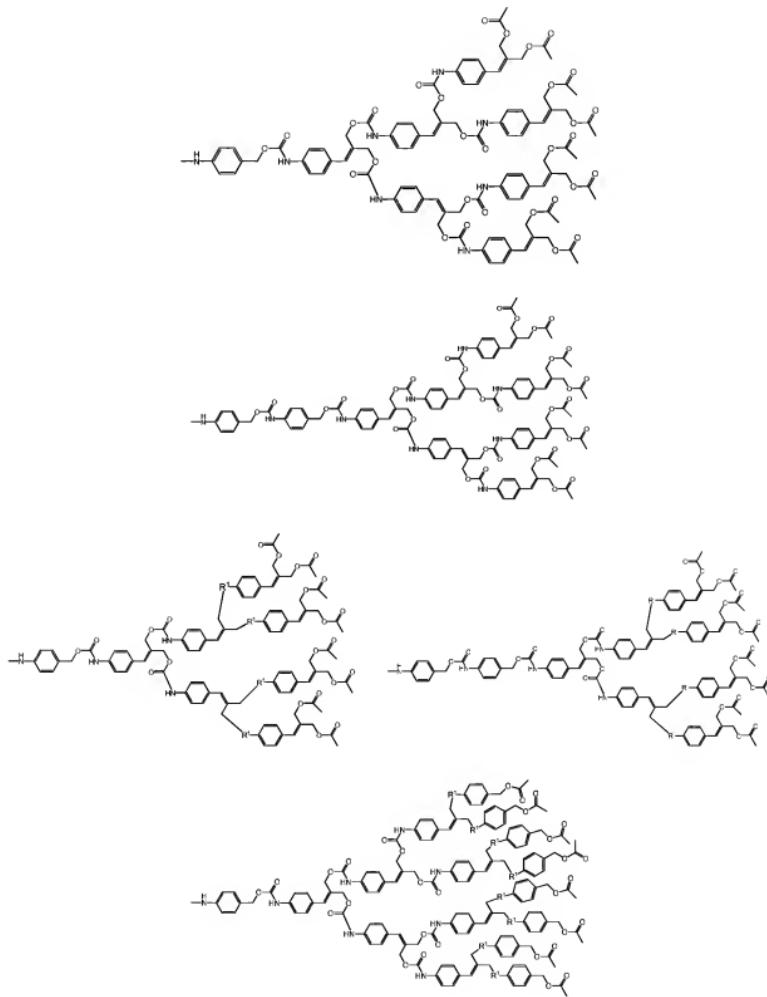
$(W)_w(X-C(D(E_e)_d)_c$ or

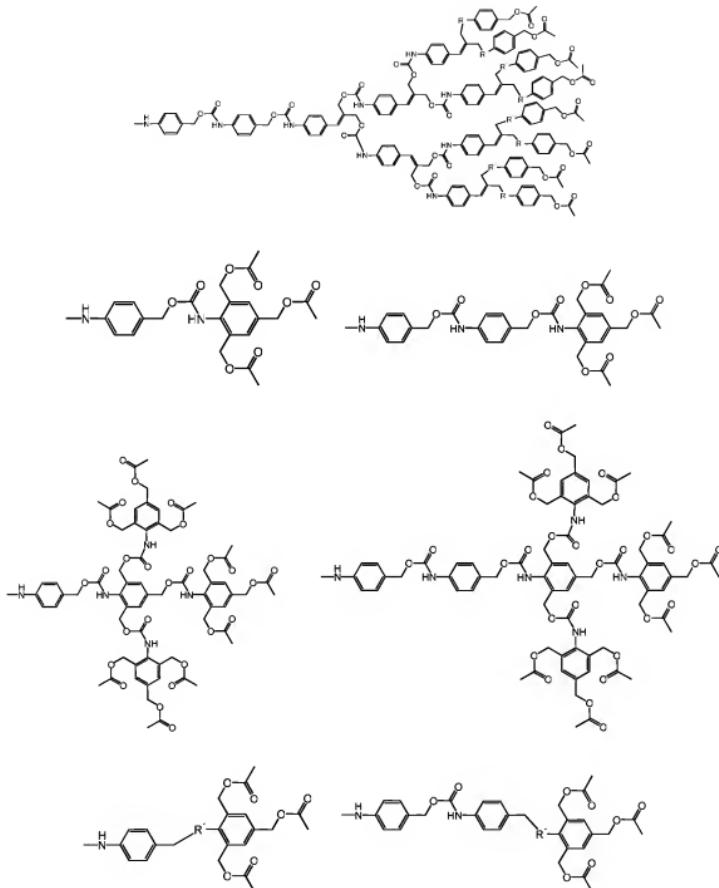
$(W)_w(X-C(D(E(F_f)_e)_d)_c$

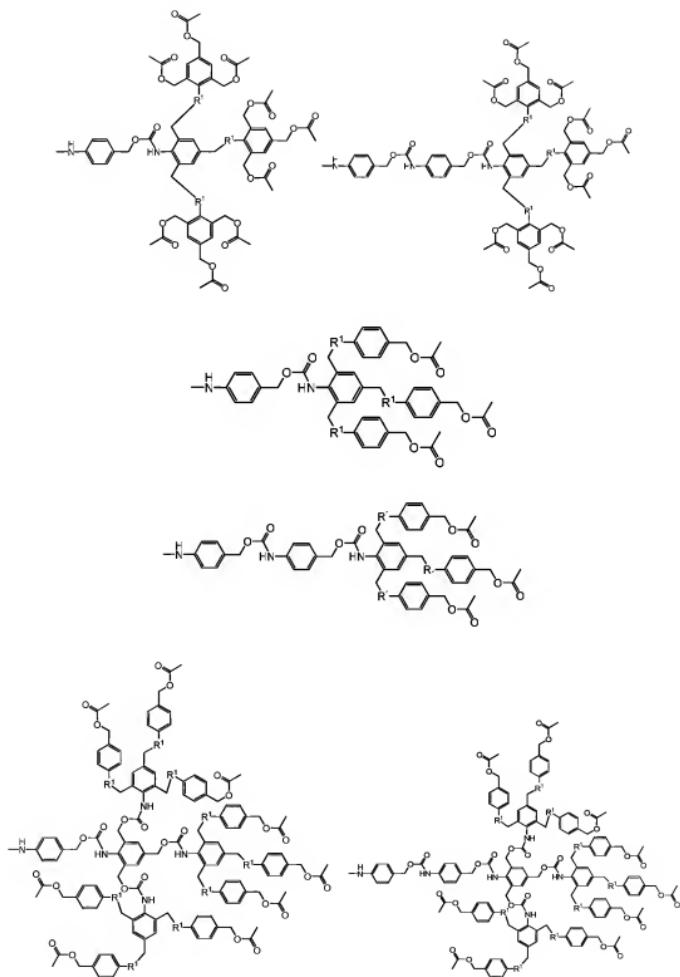
is selected from the group consisting of

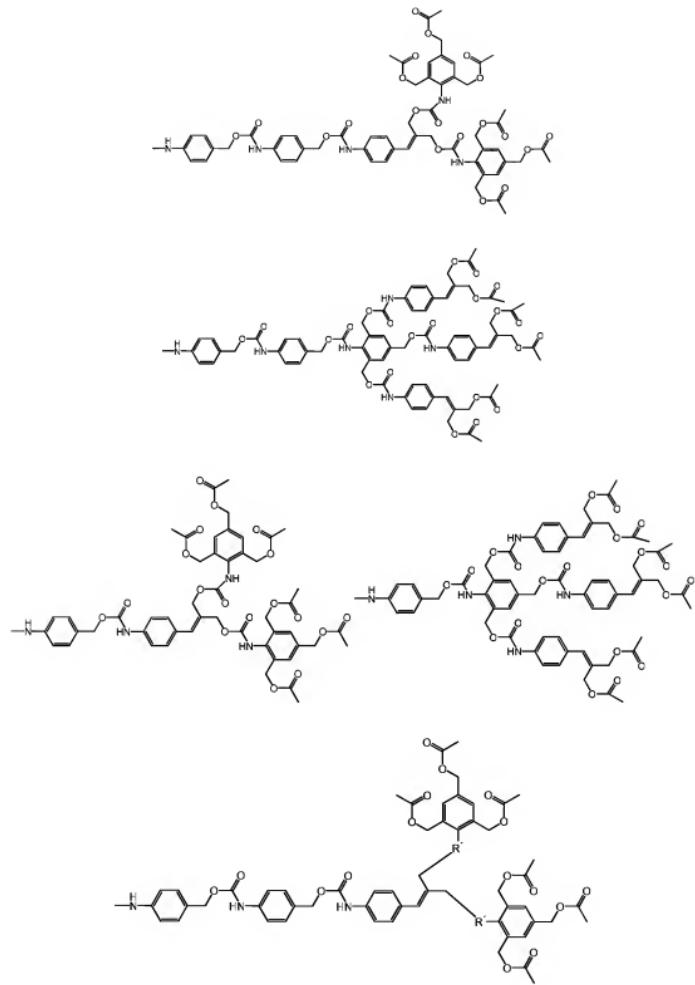


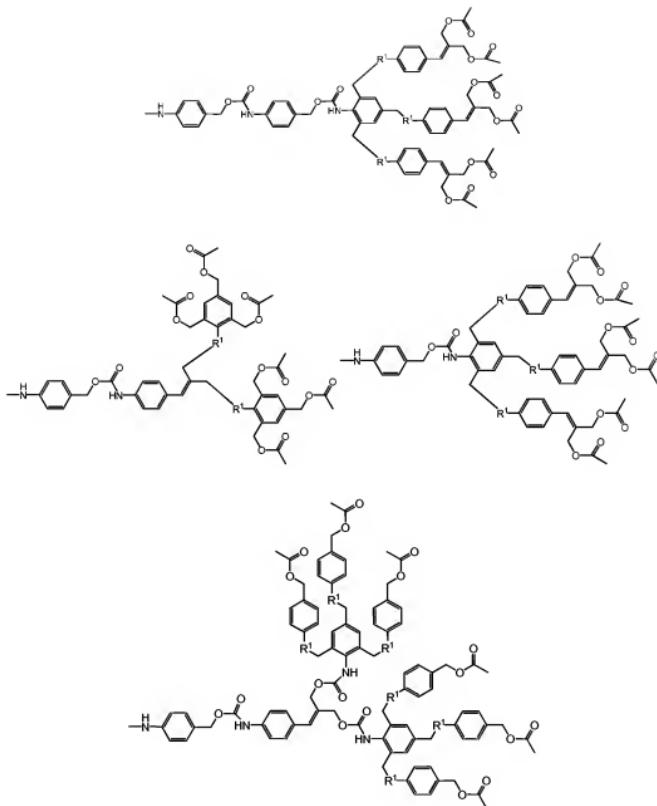


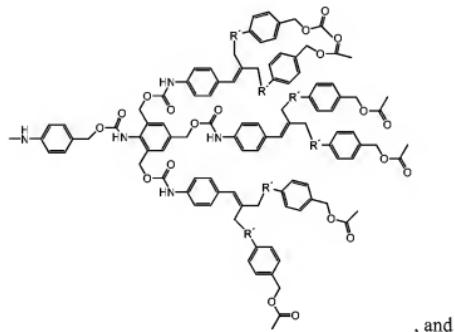
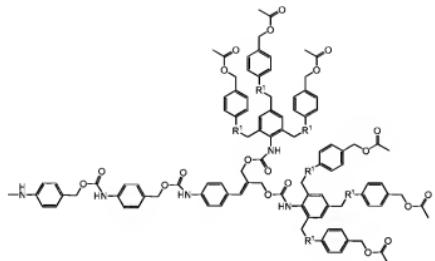




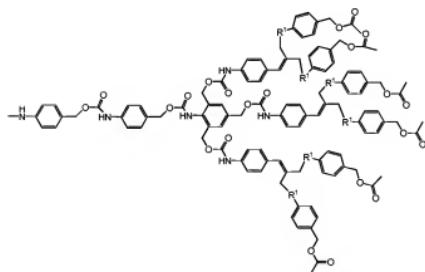








, and



; and

$\text{R}^1 = \text{OC(O)O}$.

15. (Previously presented) The compound according to claim 14, the compound further comprising cyclization elimination spacers **A**.

16. (Previously presented) The compound according to claim 4, wherein the specifier **V** contains a substrate that can be cleaved by plasmin, one of the cathepsins, cathepsin B, β -glucuronidase, prostate-specific antigen (PSA), urokinase-type plasminogen activator (u-PA), a member of the family of matrix metalloproteinases, or wherein **V-B** is an oxidized form of **B**, or wherein **V** contains a nitro-(hetero)aromatic moiety that can be removed or transformed by reduction under hypoxic conditions or by reduction by a nitroreductase.

17. (Previously presented) The compound according to claim 4, wherein **Z** is selected from an antibiotic, an anti-inflammatory agent, an anti-viral agent, and an anticancer agent.

18. (Previously presented) The compound of claim 17, wherein **Z** is selected from (hydroxyl containing cytotoxic compounds) etoposide, combrestatin, camptothecin, irinotecan (CPT-11), SN-38, topotecan, 9-aminocamptothecin, 9-nitrocamptothecin, 10-hydroxycamptothecin, GG211, lurtotecan, paclitaxel, docetaxel, esperamycin, 1,8-dihydroxy-bicyclo[7.3.1]trideca-4-ene-2,6-diyne-13-one, anguidine, doxorubicin, morpholine-doxorubicin, N-(5,5-diacetoxypentyl) doxorubicin, daunorubicin, epirubicin, idarubicin, mitoxantrone, vincristine, vinblastine, tallysomycin, bleomycin, 4-bis(2-chloroethyl)aminophenol, 4-bis(2-fluoroethyl)aminophenol, and derivatives thereof,

(sulphydryl containing compounds) esperamicin and 6-mercaptopurine, and derivatives thereof,

(carboxyl containing compounds) methotrexate, aminopterin, camptothecin (ring-opened form of the lactone), chlorambucil, melphalan, butyric acid and retinoic acid, and derivatives thereof, and

(aziridine amino containing or aromatic amino containing compounds) mitomycin C, mitomycin A, an anthracycline derivative containing an amine functionality with sufficient leaving group ability, mitoxantrone, 9-amino camptothecin, methotrexate, aminopterin,

tallysomycin, bleomycin, actinomycin, N,N-bis(2-chloroethyl)-p-phenylenediamine, N,N-bis(2-fluoroethyl)-p-phenylenediamine, deoxycytidine, cytosine arabinoside, gemcitabine, and derivatives thereof, and

(aliphatic amino containing compounds) daunorubicin, doxorubicin, epirubicin, idarubicin, N-(5,5-diacetoxypentyl)doxorubicin, an anthracycline, N⁸-acetyl spermidine, 1-(2-chloroethyl)-1,2-dimethanesulfonyl hydrazine, or derivatives thereof.

19. (Previously presented) The compound according to claim 18, wherein **Z** represents paclitaxel, docetaxel, or a derivative thereof, which is coupled to the self-eliminating multiple release spacer or spacer system via its 2'-hydroxyl group.

20. (Previously presented) The compound according to claim 18, wherein **Z** represents camptothecin, irinotecan (CPT-11), SN-38, topotecan, 9-aminocamptothecin, 9-nitrocamptothecin, 10-hydroxycamptothecin, GG211, lurtotecan, or a derivative thereof, which is coupled to the self-eliminating multiple release spacer or spacer system via its 20-hydroxyl group.

21. (Previously presented) The compound according to claim 18, wherein **Z** represents SN-38, topotecan, 10-hydroxycamptothecin, etoposide, 4-bis(2-chloroethyl)aminophenol, 4-bis(2-fluoroethyl)aminophenol, or a derivative thereof, which is coupled to the self-eliminating multiple release spacer or spacer system via its phenolic hydroxyl group.

22. (Previously presented) The compound according to claim 18, wherein **Z** represents 9-aminocamptothecin, N,N-bis(2-chloroethyl)-p-phenylenediamine, N,N-bis(2-fluoroethyl)-p-phenylenediamine, or a derivative thereof, which is coupled to the self-eliminating multiple release spacer or spacer system via its aromatic primary amine group.

23. (Previously presented) The compound according to claim 18, wherein **Z** represents daunorubicin, doxorubicin, epirubicin, idarubicin, N-(5,5-diacetoxypentyl)doxorubicin, an anthracycline, N⁸-acetyl spermidine, 1-(2-chloroethyl)-1,2-dimethanesulfonyl hydrazine, or derivatives thereof, which is coupled to the self-eliminating multiple release spacer or spacer system via its primary aliphatic amino group; wherein

at least one multiple release spacer or spacer system of either generation **C**, **D** (if present), **E** (if present), or **F** (if present) is a phenol- or thiophenol-based multiple release spacer or spacer system, meaning that

i) **B** = O or S for at least one multiple release spacer in said generation, or

ii) when **B** = N for all multiple release spacers in said generation, at least one single release spacer is connected to at least two branches of at least one multiple release spacer in said generation, and **B** = O or S for at least two of said single release spacers.

24. (Previously presented) The compound according to claim 23, wherein **B** = O or S for all multiple release spacers or spacer systems in said generation.

25. (Previously presented) The compound according to claim 23, wherein the phenol- or thiophenol-based multiple release spacers are connected to either **A** or **Z**.

26-30. (Canceled)

31. (Previously presented) The compound according to claim 4, wherein the specifier **V** is a tripeptide.

32. (Previously presented) The compound according to claim 31, wherein the tripeptide is linked via its C-terminus to the self-eliminating multiple release spacer or spacer system.

33. (Previously presented) The compound of claim 32, wherein the C-terminal amino acid residue of the tripeptide is selected from arginine and lysine, the middle amino acid residue of the tripeptide is selected from alanine, valine, leucine, isoleucine, methionine, phenylalanine, cyclohexylglycine, tryptophan and proline, and the N-terminal amino acid residue of the tripeptide is selected from a D-amino acid residue and a protected L-amino acid residue including protected glycine.

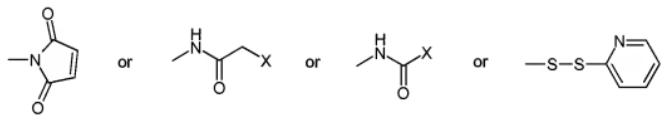
34. (Previously presented) The compound according to claim 33, wherein the specifier **V** is selected from D-alanylphenylalanyllysine, D-valylleucyllysine, D-alanylleucyllysine, D-valylphenylalanyllysine, D-valyltryptophanyllysine and D-alanyltryptophanyllysine.

35. (Previously presented) The compound according to claim 4, wherein the specifier V is an amino-terminal capped peptide covalently linked via the C-terminus to the self-eliminating multiple release spacer or spacer system.

36. (Previously presented) The compound according to claim 35, wherein the specifier V is selected from benzyloxycarbonylphenylalanyllysine, benzyloxycarbonylvalyllysine, D-phenylalanylphenylalanyllysine, benzyloxycarbonylvalylcitrulline, tert-butyl oxycarbonylphenylalanyllysine, benzyloxycarbonylalanylarginylarginine, benzyloxycarbonylphenylalanyl-N-tosylarginine, 2-aminoethylthiosuccinimidopropionylvalinylcitrulline, 2-aminoethylthiosuccinimidopropionyllysylphenylalanyllysine, acetylphenylalanyllysine, and benzyloxycarbonylphenylalanyl-O-benzoylthreonine.

37. (Previously presented) The compound according to claim 4, wherein the specifier V comprises a reactive moiety that can be used to couple said compound to a targeting moiety.

38. (Previously presented) The compound according to claim 37, wherein the reactive moiety is



wherein X is a leaving group.

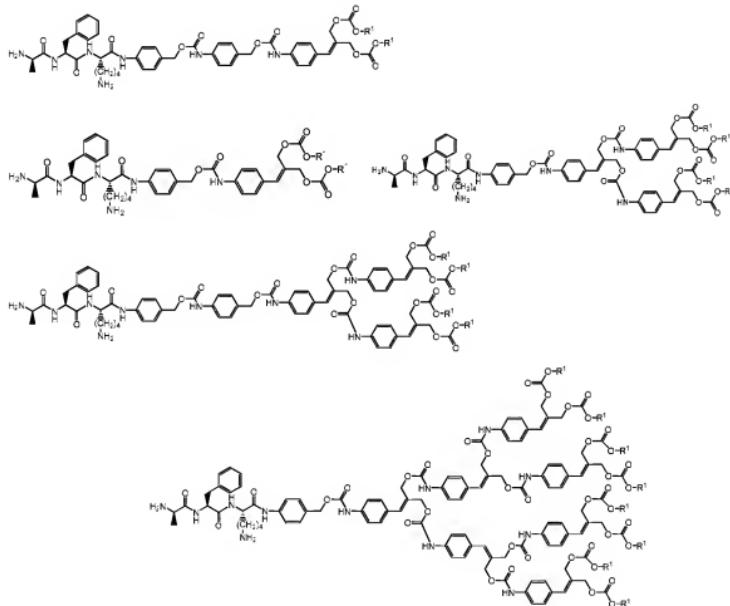
39. (Previously presented) The compound according to claim 37, wherein the reactive moiety is selected from an *N*-hydroxysuccinimide ester, a *p*-nitrophenyl ester, a pentafluorophenyl ester, an isothiocyanate, an isocyanate, an anhydride, an acid chloride, a sulfonyl chloride, and an aldehyde.

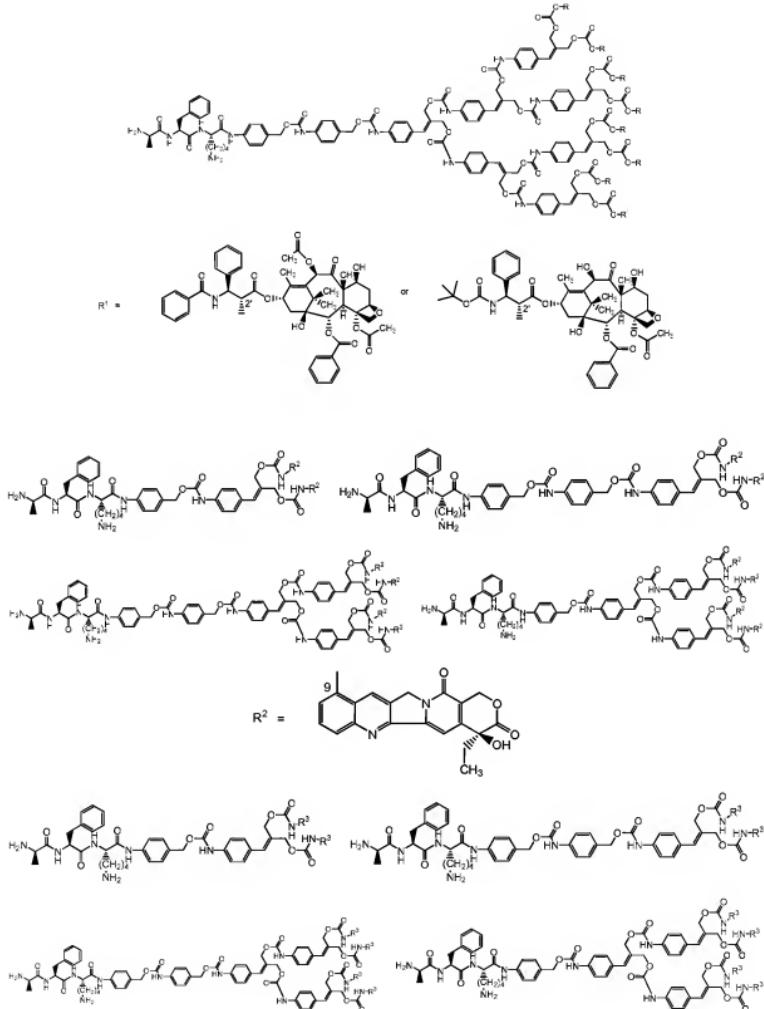
40. (Previously presented) The compound according to claim 37, wherein the reactive moiety is a hydrazine group or an amino group.

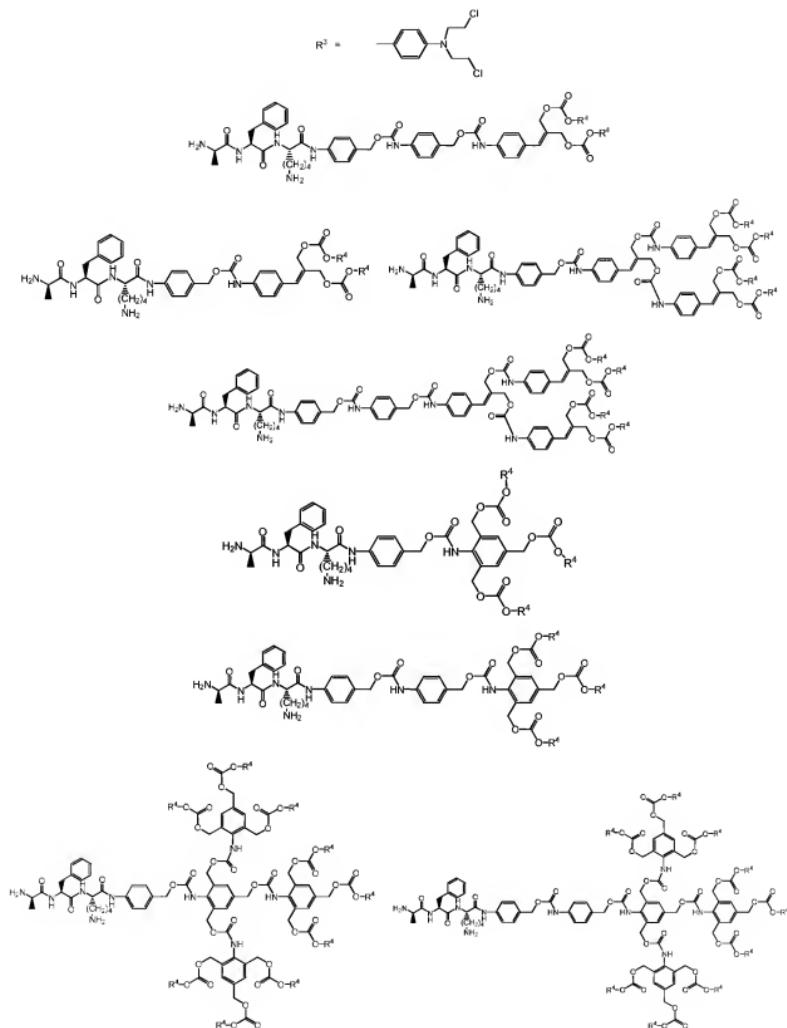
41. (Previously presented) The compound according to claim 4, wherein the specifier V comprises a targeting moiety.

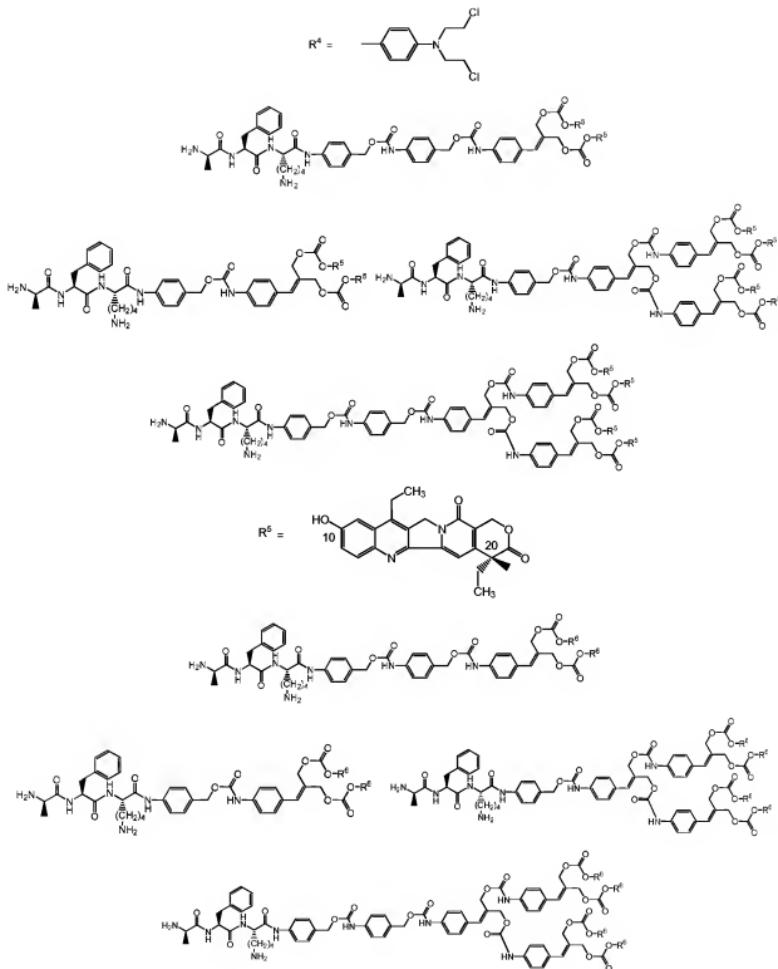
42. (Previously presented) The compound according to claim 41, wherein the targeting moiety is selected from the group consisting of a protein or protein fragment, an antibody or an antibody fragment, a receptor-binding or peptide vector moiety and a polymeric or dendritic moiety.

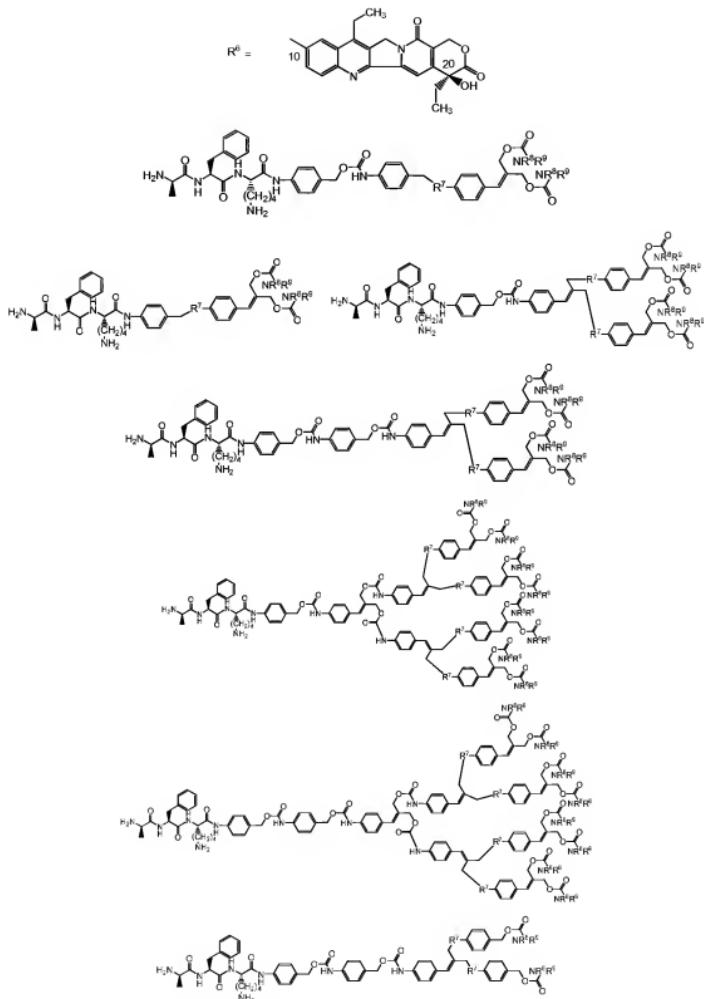
43. (Previously presented) A compound selected from the group consisting of

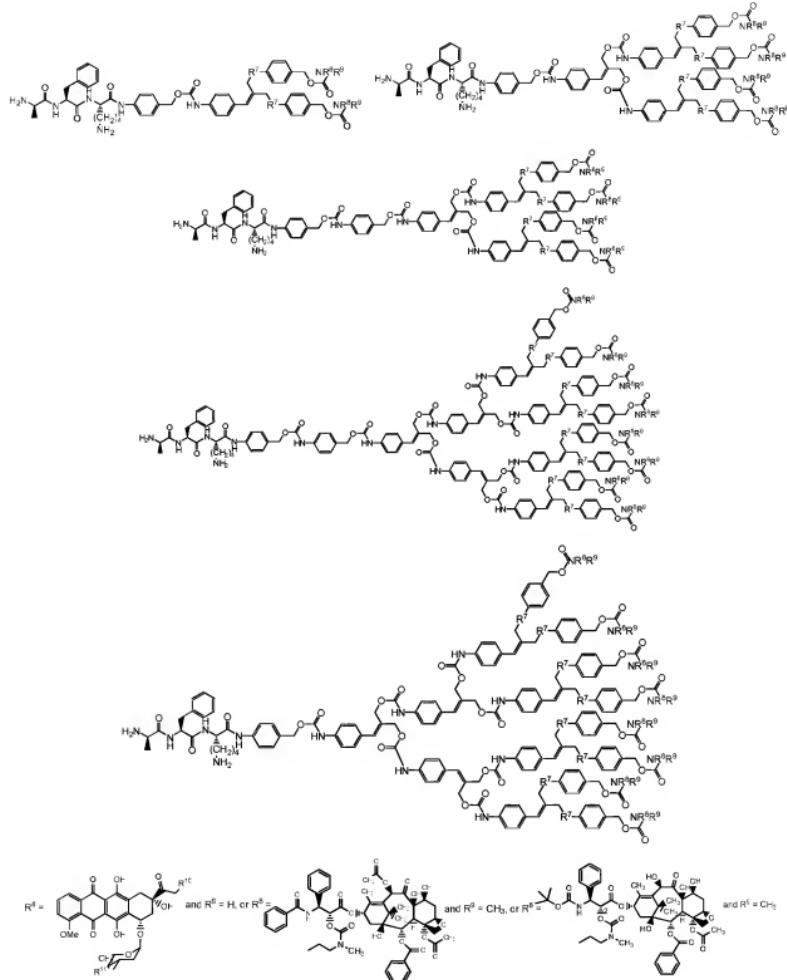












and salts thereof, wherein R⁷ = OC(O)O.

44. (Canceled)

45. (Previously presented) A diagnostic assay process, the process comprising: incubating a sample comprising an enzyme with a compound according to claim 4 to cause enzymatic cleavage of the compound, and detecting one or more molecules liberated by the enzymatic cleavage.

46. (Previously presented) The diagnostic assay process according to claim 45, wherein the detection of the one or more molecules determines the presence or amount of the enzyme

47. (Previously presented) The diagnostic assay process according to claim 46, wherein the detection of the one or more molecules determines the presence or amount of a protease.

48. (Previously presented) The diagnostic assay process according to claim 47, wherein the compound that is used comprises a substrate for said protease and one or more Z groups are detected.

49. (Previously presented) The diagnostic assay process according to claim 47, wherein the compound that is used comprises a substrate for the enzyme, which is the product of cleavage of its pro-enzyme precursor by said protease and one or more Z groups are detected.

50. (Previously presented) A composite structure comprising two or more compounds according to claim 4 connected with a polymeric structure.

51. (Previously presented) The compound according to claim 4, wherein the specifier V can be removed or transformed by an enzyme that is transported to the vicinity of or inside target cells or target tissue via ADEPT, PDEPT, MDEPT, VDEPT, or GDEPT.

52. (Canceled)

53. (Previously presented) A pharmaceutical composition comprising a compound according to claim 4.

54. (Previously presented) A process for preparing a pharmaceutical composition comprising the step of mixing a compound according to claim 4 with a pharmaceutically acceptable carrier.

55. (Cancelled)